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Environment and primary biliary cirrhosis: Electrophilic drugs and the induction of AMA

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ABSTRACT

Environmental stimulation is a major factor in the initiation and perpetuation of autoimmune diseases. We have addressed this issue and focused on primary biliary cirrhosis (PBC), an autoimmune disease of the liver. Immunologically, PBC is distinguished by immune mediated destruction of the intra hepatic bile ducts and the presence of high titer antimitochondrial autoantibodies (AMA) directed against a highly specific epitope within the lipoic acid binding domain of the pyruvate dehydrogenase E2 subunit (PDC-E2). We submit that the uniqueness of AMA epitope specificity and the conformational changes of the PDC-E2 lipoyl domain during physiological acyl transfer could be the lynchpin to the etiology of PBC and postulate that chemical xenobiotics modification of the lipoyl domain of PDC-E2 is sufficient to break self-tolerance, with subsequent production of AMA in patients with PBC. Indeed, using quantitative structure activity relationship (QSAR) analysis on a peptide-xenobiotic conjugate microarray platform, we have demonstrated that when the lipoyl domain of PDC-E2 was modified with specific synthetic small molecule lipoyl mimics, the ensuing structures displayed highly specific reactivity to PBC sera, at levels often higher than the native PDC-E2 molecule. Hereby, we discuss our recent QSAR analysis data on specific AMA reactivity against a focused panel of lipoic acid mimic in which the lipoyl di-sulfide bond are modified. Furthermore, data on the immunological characterization of antigen and Ig isotype specificities against one such lipoic acid mimic; 6,8-bis(acetylthio)octanoic acid (SAc), when compared with rPDC-E2, strongly support a xenobiotic etiology in PBC. This observation is of particular significance in that approximately one third of patients who have taken excessive acetaminophen (APAP) developed AMA with same specificity as patients with PBC, suggesting that the lipoic domain are a target of APAP electrophilic metabolites such as NAPQI. We submit that in genetically susceptible hosts, electrophilic modification of lipoic acid in PDC-E2 by acetaminophen or similar drugs can facilitate loss of tolerance and lead to the development of PBC.

1. Molecular mimicry and xenobiotics in autoimmune diseases

Accumulating data from epidemiological studies on autoimmunity have strongly implicated the role of environment in the etiology [1,2]. In autoimmunity, the paradox is that autoantigens are unable to elicit a primary immune response themselves but can be recognized as targets for effector T cells stimulated by a pathogenic cross-reactive epitope. To break self-tolerance to the autoantigen, the epitope mimic or mimetope needs to induce activation and proliferation rather than anergy of autoreactive T cells. Subsequently, the autoantigen presented by the host cells of a certain tissue must be recognized by reactive epitope-specific T cells to

Abbreviations: AMA, antimitochondrial autoantibodies; ALF, acute liver failure; APAP, acetaminophen; BCOADC-E2, E2 subunits of branched chain 2-oxo acid dehydrogenase complex; OADC-E2, E2 subunits of the 2-oxo-acid dehydrogenase complexes; OGDC-E2, E2 subunits of 2-oxo-glutarate dehydrogenase complex; OASAc, 8-(acetylthio)octanoic acid; PBC, primary biliary cirrhosis; PDC-E2, E2 subunits of pyruvate dehydrogenase; QSAR, quantitative structure activity relationship; NAPQI, N-acetyl-p-benzoquinoneimine; SAc, 6,8-bis(acetylthio)octanoic acid; SCOEt, 6,8-bis(propionylthio)octanoic acid; TFA, trifluoroacetylated.

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cause autoimmune disease. Among the hypothetical mechanisms for the environmental etiology of autoimmunity, the concept of self-antigen changes induced by chemical and infectious agents which could break tolerance by post-translational modifications and molecular mimicry have received substantial attention [3]. This mechanism of molecular mimicry has been suggested to be associated with several systemic autoimmune diseases, including multiple sclerosis [4–6], systemic lupus erythematosus (SLE) [7,8] and rheumatoid arthritis [9–11].

Although bacteria and viruses are candidates for the induction of autoimmune disease by molecular mimicry [12], there are also other environmental factors, xenobiotics or chemical compounds foreign to a living organism. Examples include drugs, pesticides or other organic molecules that have the potential to modify host proteins and render them more immunogenic [2]. Halothane hepatitis is a xenobiotics-induced liver disease that occurs when susceptible individuals develop an immune response against trifluoroacetylated (TFA) protein antigens. Exposure to TFA-conjugated self-proteins results in antibody responses against such TFA-self proteins. Interestingly, the human PBC mitochondrial autoantigen; lipoylated inner lipoyl domain of PDC-E2, but not the unlipoylated form, is also recognized by anti-TFA [13]. Here, we will first provide an overview of the natural history, genetics and immunobiology of PBC. We will also discuss the biochemistry of PDC-E2 and its potential susceptibility to xenobiotic modifications, with particular emphasis on our recent data supporting that xenobiotic modification of lipoyl-PDCE2 and finally our hypothesis on the role of electrophilic drugs modification of PDC-E2 in breaking of tolerance in PBC.

2. Natural history and genetics of primary biliary cirrhosis

Primary biliary cirrhosis (PBC) is a liver specific autoimmune disease. The incidence of PBC is 2.7 per 100,000 in a well defined US population [14] but varies between geographic locations [15,16]. PBC is more prevalent in Northern Europe and North America and less common in Eastern Asia, Africa, and Australia [17,18]. Epidemiological studies suggest that the incidence of PBC is increasing [15]; it affects women predominantly with a female:male ratio of 9:1 and a middle age onset [19]. PBC is characterized by the presence of high titer antimitochondrial autoantibodies (AMA) and immune mediated progressive destruction of biliary epithelial cells (BEC) of small bile ducts, eventually leading to cholestasis, fibrosis and potentially liver cirrhosis [19]. Approximately 50–60% of patients are asymptomatic at diagnosis and the disease has a long latency period [20,21], followed by the development of symptoms, usually fatigue, pruritus, hyperpigmentation of the skin, and later bleeding varices, edema, or ascites [22]. The prognosis of patients diagnosed with PBC has improved significantly over the past 2 decades perhaps because patients are being diagnosed earlier.

The female predominance in PBC suggests there are significant genetic components in this disease, supported by the high frequency of monosomy of the X chromosome in PBC [23]. Reports from recent genetic studies demonstrate that in addition to the HLA-locus, several genetic susceptibility loci of IL12-related pathways, SPIB, IRF5-TNPO3, and 17q12-2 are associated with PBC; new candidate genes include STAT4, DENND1B, CD80, IL7R, CXCR5, TNFRSF1A, CLEC16A, and NFKB1 have been identified by genome wide association studies [24–27]. Data on familial clustering of PBC demonstrates that first-degree relatives of PBC patients have an increased risk of developing disease. Most often, these familial clusters involve mother–daughter pairs, which is consistent with the female preponderance of the disease [28,29]. Furthermore, twin studies have demonstrated a high concordance for PBC in monozygotic twins and a low concordance among dizygotic twins [30]. These clusters provide evidence for a genetic basis underlying PBC. On the other hand, clusters of nonrelated individuals suggest that environmental factors also play a role in the development of the disease [31]. Environmental components including chemicals [32–34] have been implicated in initiating PBC.

The understanding that environment plays a critical role in the development of autoimmunity was based in part on studies of concordance of specific autoimmune diseases in identical twins [35]. It is interesting to note that with the exception of PBC, the penetrance of autoimmunity in monozygotic twins ranges typically between 20 and 30%, suggesting that factors gene sequence itself is not sufficient in determining the outcome of autoimmunity [36]. Moreover, the concordance rate of multiple autoimmune diseases in monozygotic twins such as rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, type I diabetes mellitus and psoriasis also varies considerably [3,36,37]. This lack of concordance in monozygotic twins in autoimmune diseases strongly suggests that environmental and/or epigenetic factors are also important in determining the susceptibility to autoimmunity. The thesis is further supported by the observation from large scale single polymorphism analysis multicenter studies and genome wide association studies that significant genetic associations between certain SNPS and autoimmunity are only limited to certain subgroups of patients [38,39]. Compelling data from epidemiologic studies also highlighted the contribution of environmental factors in the development of human autoimmune diseases [1,2]. The need to dissect various environmental stimuli (e.g. chemicals, toxins, drugs, pharmaceuticals, ultraviolet radiation) and their mechanisms in breaking of tolerance is eminent [2]. Given the higher incidence of autoimmunity in female [40], it will be of interest to understand how different stimuli may differentially affect males and females and the biological pathways involved [41]. Data from animal models have led to increased confidence about environmental factors that affect expression of autoimmunity [42] and constitute extremely valuable tools for studying the induction or exacerbation of autoimmunity by environmental conditions and exposures.

3. Immunobiology of primary biliary cirrhosis

AMA is present in over 95% of patients with PBC and is the most specific diagnostic antibody marker of PBC [43]. The autoantigens of AMA have been identified as the E2 subunits of the 2-oxo-acid dehydrogenase complexes (2OADC-E2), including the E2 subunits of the pyruvate dehydrogenase complex (PDC-E2), branched chain 2-oxo acid dehydrogenase complex (BCOADC-E2) 2-oxo-glutarate dehydrogenase complex (OGDC-E2) [44–47] and the E3 binding protein of dihydrolipoamide dehydrogenase [48]. The AMA target antigens are all localized within the inner mitochondrial matrix and catalyze the oxidative decarboxylation of 2-oxo-acid acid substrates [49]. Biochemically, 2OADC-E2 have a common functional domain containing a single or multiple lipoyl groups. The immunodominant epitopes recognized by AMA are all mapped within the lipoyl domains of these target antigens [50,51]. In patients with PBC, T helper ($CD4^+$) T cells and cytotoxic ($CD8^+$) T cells are present in portal tracts, around damaged bile ducts [19]. Although PDC-E2 autoreactive CD4 T cells are present in peripheral blood and liver; there is a specific 100–150 fold increase in number of PDC-E2-specific CD4 T cells in the hilar lymph nodes and liver versus peripheral blood in patients with PBC [52]. Similar to CD4 autoreactive T cells, there is a 10-fold higher frequency of PDC-E2 specific CD8 T cells within the liver versus peripheral blood. Moreover, the precursor frequency of PDC-E2-specific autoreactive CD8 T cells is significantly higher in early rather than late stage of the disease [53]. The PDC-E2 autoepitope for both CD4 and CD8 T cells, overlaps with the B cell epitope, which spans the lipoyl domain [53]. Recent reports also substantiate the involvement of innate immunity, including monocytes, toll like